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RESEARCH ARTICLE

Clopidogrel as an Oral Antiaggregant in Ischemic Heart Disease

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Abstract

The use of antiaggregant in preventing cardiovascular diseases remains an important therapeutic approach. The research aims to perform a comparative analysis of the effect of acetylsalicylic acid (aspirin) and clopidogrel monotherapy or their combination on platelet aggregation applying the method of impedance aggregometry. The study was carried out in 2018-2019 in Grozny (Russia) and in Ho Chi Minh (Vietnam) based on the past medical histories of 557 patients diagnosed with ischemic heart disease in 3 primary health-care facilities. The acetylsalicylic acid monotherapy in amount 100 mg per day was used for the treatment of 367 patients comprising Group I, another 147 patients (Group II) received clopidogrel in a dosage 75 mg per day, and the rest 43 patients (Group III) took both drugs. The method of impedance aggregation with the estimation of three parameters, such as platelet aggregation time, aggregation amplitude, and the area under the aggregation curve, was applied after the introduction of two inducers, namely, collaagen and ADP. In Group I, the use of collagen as an inducer resulted in prolonged platelet aggregation period and reduction of area under the curve (correlation -0.80 ($p \leq 0.05$)). In Group II, the influence of clopidogrel on both inducers was reported. In the case of ADF-initiated platelet aggregation, a positive correlation between amplitude and area under the aggregation curve of 0.98 ($p \leq 0.01$) was recorded. A correlation of 0.77 ($p \leq 0.05$) was noted between the platelet aggregation extension and the area under the collagen-induced process curve. The results obtained through this research in the opposite correlation coefficients for Group I and Group II show the mechanisms of acetylsalicylic acid and clopidogrel action. The applied methodology allowed establishing qualitative and quantitative changes occurring in the aggregation of platelets with the addition of ADP and collagen as inducers.

Keywords: *ADP; Antiaggregant; Acetylsalicylic acid (ASA); Clopidogrel; Collagen; Ischemic heart disease.*

Introduction

Diseases of the cardiovascular system are one of the main reasons for the high mortality rate. According to numerous research data, half of all fatal cases among patients suffering from these illnesses are due to ischemic heart disease (IHD) [1].The main cause for death is myocardial infarction developing as a result of thrombosis processes. Thrombocytes play a key role in the atherothrombotic process [2].Therefore, the use of antiaggregant as preventive tactics remains highly relevant today. Acetylsalicylic acid (aspirin) or ASA remains the most widely used drug in medical and daily practice, and its effect manifests in reducing platelet aggregation. Antiagregants used in modern pharmacotherapy vary significantly in the mechanism of action [3].ASA belongs to the medications that inhibit enzyme cyclooxygenase activity (COX-1), the rest are considered as P2Y12 receptors blocking adenosine diphosphate (ADP). This group includes drugs of the first generation (ticlopidine), the second generation (cangrelor, clopidogrel), and the third generation (prasugrel, ticagrelor). Preparations blocking glycoprotein receptors GPIIb/IIIa like abciximab, eptifibatide, and tirofiban are applied as well. The action of clopidogrel is confined to the metabolite

formation that irreversibly binds two components-ADP itself and P2Y12 receptors. At that, in thrombocytes, the receptors of GPIIb/IIIa complex are activated, the number of active ADP receptors decreases, as well as the thrombus formation components - thromboxane and collagen, and fibrinogen protein sorption. The initial dose of clopidogrel amounts to 300 mg with achieving an antiaggregant effect within 30-60 min [4].

Existing studies compare the efficacy and safety of second-generation drugs like clopidogrel and third-generation drugs like prasugrel and ticagrelor [5, 9]. For example, the TRITON study [10] involved patients with the acute coronary syndrome at a ratio of 74% (patients without ST ascension) to 24% (patients with ST ascension).

An initial dosage of 300 mg per day was prescribed to patients undergoing clopidogrel therapy with subsequent reduction to 75 mg per day. The second group included patients under treatment with prasugrel at the initial dose of 60 mg and the following reduction to 10 mg per day. All patients were on medication for 6-15 months. Cumulative risk assessment, i.e., the incidence of nonfatal myocardial infarcts and other cardiovascular events, was performed.

The results were evaluated regardless of the type of acute coronary syndrome. Higher risk results were obtained for those receiving clopidogrel (12.1% of cases) and prasugrel (9.9% of cases) therapy. Treatment with prasugrel showed good results for patients with stent coronary arteries. At that, the risk of thrombosis reduced with a significant difference of 1.1% and 2.4% by clopidogrel therapy. However, all the advantages of prasugrel were offset by a very high risk of complications like large, spontaneous, and fatal bleedings. The difference against clopidogrel was 0.6, 0.5, and 0.3 % respectively.

The use of prasugrel was estimated as especially dangerous for elderly people as well as for people with low body mass index (up to 60 kg). For them, the risk of bleeding prevailed over the therapeutic effect. It has been also established that prasugrel should not be prescribed to persons who had undergone a transit ischemic attack. Another study, PLATO, compared the efficacy of ticagrelor and clopidogrel [11].

The ratio of patients with and without ST ascension was 41% and 59%, respectively. Invasive and non-invasive therapies were exercised among the patients. The efficiency of treatment with ticagrelor was found to be higher, which was reflected in a decreasing number of strokes, myocardial infarcts, and other cardiovascular mortality factors up to 9.8% as compared to 11.7% in the clopidogrel group.

A slightly higher probability of heavy bleeding and dyspnea (13.8% and 7.8%, respectively) was found for ticagrelor medication in comparison with clopidogrel. This contributes to restricting the usage of the drug for patients with chronic lung diseases like bronchitis, tracheitis, etc. The efficacy of clopidogrel has been proven by numerous studies on a large sample of patients. The first such study was CAPRIE [12].

The study enrolled 19.185 patients with cardiovascular complications and aimed to evaluate the clopidogrel efficacy versus ASA treatment. The mortality rate within the clopidogrel group was found to be 8.7% lower in comparison to patients receiving ASA. Besides, the risk of ischemic stroke was reduced by 7.3% while taking clopidogrel. By extensive atherothrombosis, the efficacy of the clopidogrel therapy was doubled up to 15.4%.

This medication also had less pronounced side effects compared to that by ASA intake. The follow-up CAPRIE study after CAPRA research [13] clarified the number of patients who required clopidogrel treatment for a single cardiovascular episode. As a result, the recommended number of patients was reduced from 200 to 70. Clopidogrel was also found to have an advantage over ASA in patients with recurrent multiple vascular lesions. Clopidogrel is known to have a positive effect compared to ASA in patients diagnosed with diabetes, ischemic stroke, and myocardial infarction [1].

The aforementioned facts indicate the relative efficacy of clopidogrel therapy in comparison with ASA or with the medications of the next generation. First of all, it concerns the danger of bleeding. Despite a large number of studies on pharmacokinetics, pharmacology, and biochemistry of clopidogrel [3], such issues as

comparing the morpho-functional state of platelets by clopidogrel or ASA monotherapy and by combination therapy with these drugs remain highly relevant. Practical studies of patients' sensitivity to antiagregants are required. The authors assume that methods of impedance aggregation can be employed to determine the effect of antithrombotic drugs on platelet aggregation in patients with circulatory disorders.

Aim

The purpose of this work is to perform a comparative analysis of the antiaggregant drugs' effect on platelet aggregation during ASA and clopidogrel monotherapy or in combination therapy with these drugs based on data obtained by impedance aggregation. The objective of the study is to evaluate the efficacy of each drug on platelet aggregation and to identify possible morpho-functional changes in platelets in each type of therapy. The study was conducted in 2018-2019 in Grozny (Russia) and in Ho Chi Minh (Vietnam). A total of 557 anamneses of patients with the diagnosis of IHD from 3 health-care facilities were analyzed.

Of these patients, 367 received ASA as an antiaggregant (Group I, 65.8%), 147 took clopidogrel (Group II, 26.3%), and the rest 43 were assigned with both drugs (Group III, 7.9%). Antiaggregants were taken according to standard recommendations, namely, 100 mg of ASA and 75 mg of clopidogrel per day. As secondary prevention, patients took statins as well as antihypertensive drugs in standard dosages. The average ages of patients, as well as their distribution by gender, are given in Table 1.

Table 1: Distribution of patients by gender and age

Group	Male patients, number of patients, %	Average age, years	Female patients, number of patients, %	Average age, years
I (acetylsalicylic acid)	198 (53.9)	61.0±0.2	169 (46.1)	60.2±0.3
II (clopidogrel)	78 (53.0)	60.5±0.1	69 (47.0)	61.8±0.2
III (acetylsalicylic acid + clopidogrel)	21 (48.8)	62.4±0.4	22 (51.2)	60.7±0.3

As can be seen from the data in Table 1, no significant differences in distribution between gender and the average age groups were reported

Material and Methods

Thrombocyte activity in patients of all three groups was evaluated using the method of impedance aggregometry. For this purpose, the apparatus CHRONO-LOG 590 (USA) was employed. Whole blood was selected for the study, and collagen and ADP were analyzed as inducers.

The following inducers concentrations were taken as the final indicators -2 μg per 1 ml for collagen and 10 μm per 1 ml for ADP. As a result of the analysis, the curve of changes in electrical potential indicators was obtained. The result of aggregation in a patient not taking antiagregants is given as an example in Figure 1.

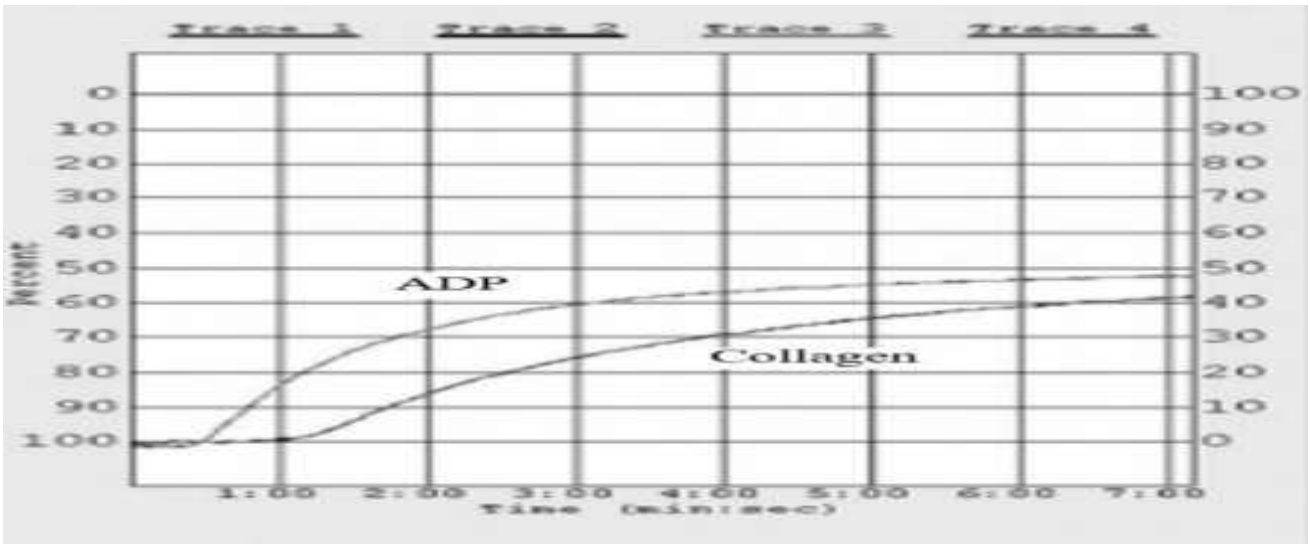


Figure 1: Impedance aggregometry in patients without antiaggregant intake

After results were established, the following three parameters were evaluated. First, the Lag Time or the time interval between the injection of the inducer and the initiation of the platelet assembly process. These processes are interconnected, i.e., the later the initiation of the aggregation process begins, the lower the platelet activity. This parameter is measured in seconds.

The second parameter is the amplitude of aggregation, measured in ohm. It displays the highest values of change in electrical potential and aggregation in response to the inducer. The third parameter is the area under the aggregation curve integrally showing the platelet activity, measured in ohm*sec. Thrombocyte aggregation is associated with the second and third parameters in a direct correlation -the less pronounced it is, the lower the values in the second and third parameters.

The results of aggregometry obtained for patients from all three groups were further compared with those used as a standard in the clinical and diagnostic department of the clinic.Besides, lipid profile parameters, concentration level of C-reactive protein, fibrinogen, and D-dimer were evaluated.

For electron microscopy, 10 ml of whole blood was taken from 15 IHD patients not receiving antiaggregants and not included in any of the three groups. Afterward, the blood samples were fixed in a glutaric aldehyde solution of 2.5% concentration. The fixation time was 1.5 hours at room temperature (21 ^{o}C). The sample was then placed on a porous polycarbonate matrix with each pore size of 0.22 nm in diameter with subsequent dehydration, drying, and spraying.

The photographs were taken with a PHILLIPS PSEM 550× scanning electron microscope. In this case, it was necessary to determine what kind of changes occurs in blood at clotting.

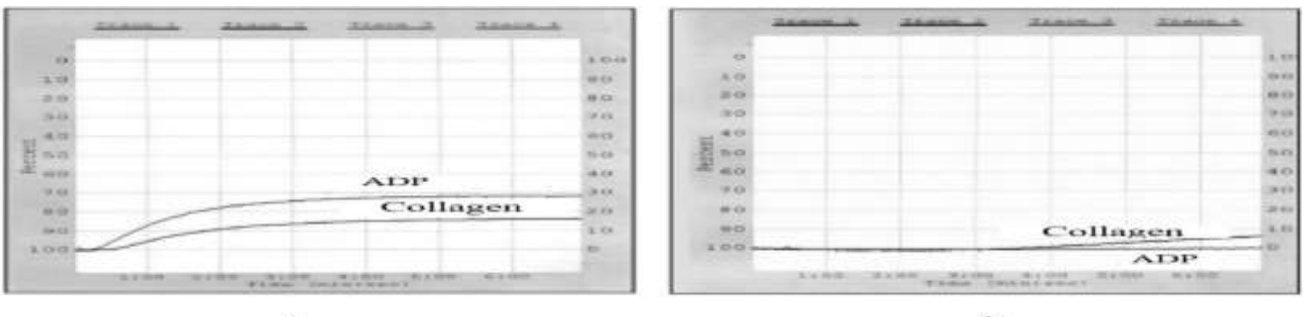
Statistical analysis

The statistical processing of data was performed in Statistica 6.0 software (Stat Soft Inc.). The data arrays were checked with the Kolmogorov-Smirnov test for normality, and then the arithmetic mean for each indicator was calculated, as well as the error in the mean. In case when distribution differs from the normal, the median method from 25 to 75 quartiles was applied.

For adequate estimation of results obtained from different categories, the values of absolute frequencies and the percentages expressed from the total number of performed observations were calculated. In order to compare the influence degree of antiaggregant therapy in groups, single factor dispersion analysis (ANOVA) was applied, and for a distribution different from the normal, the Cruckele-Wallis method was used. Pearson's correlation coefficient was calculated to establish the strength of the interconnection between quantitative parameters.

Results

Application of the impedance aggregation method allowed establishing a number of regularities during platelet aggregation process in patients from different groups. Thus, in Group I receiving ASA, the beginning of the aggregation in thrombocytes was lengthened to 74.1 ± 1.0 sec with a decrease of the area under the aggregation curve to 53.6 ± 0.9 ohm * sec. An inverse relationship between these parameters can be observed, namely, the Pearson's correlation coefficient was -0.80 ($p \leq 0.05$) (Figure 2*a*).



a b

Figure 2: *a*) Response to ASA intake in a patient of Group I with reduction of platelet aggregation initiated by collagen; *b*) Response to clopidogrel intake in a patient of Group II with reduction of platelet aggregation initiated by ADP

While clopidogrel reception, a different result was observed, namely, a decrease in platelet aggregation initiated by ADP. This is due to the blockage of ADP receptors in thrombocytes by clopidogrel medication. The thrombocyte aggregation amplitude decreased to 8.7 ± 0.9 ohm and the area under the aggregation curve reduced to 37.9 ± 4.1 ohm*sec.

In contrast to the obtained negative correlation between aggregation parameters in patients of Group I, a positive correlation between amplitude and the area under the aggregation curve of 0.98 ($p \leq 0.01$) was noted in Group II. At the same time, a decrease in platelet aggregation associated with collagen was noticed, in particular, it prolonged to the level of 76.7 ± 5.5 sec as in the case of ASA intake in Group I. Also, the area under the aggregation curve was reduced to the values of 53.4 ± 2.7 ohm*sec. The inverse correlation of 0.77 ($p \leq 0.05$) was recorded for these two indices. For patients from Group II, the significant difference in three considered parameters was not observed (Figure 3), which can be attributed to a small sample size.

In general, platelet aggregation indices initiated by ADP tend to reduction, namely, the amplitude and area under the aggregation curve. Also, a decrease in platelet aggregation caused by collagen was reported followed by a decrease in LOG Time values and area under the aggregation curve (Figure 3).

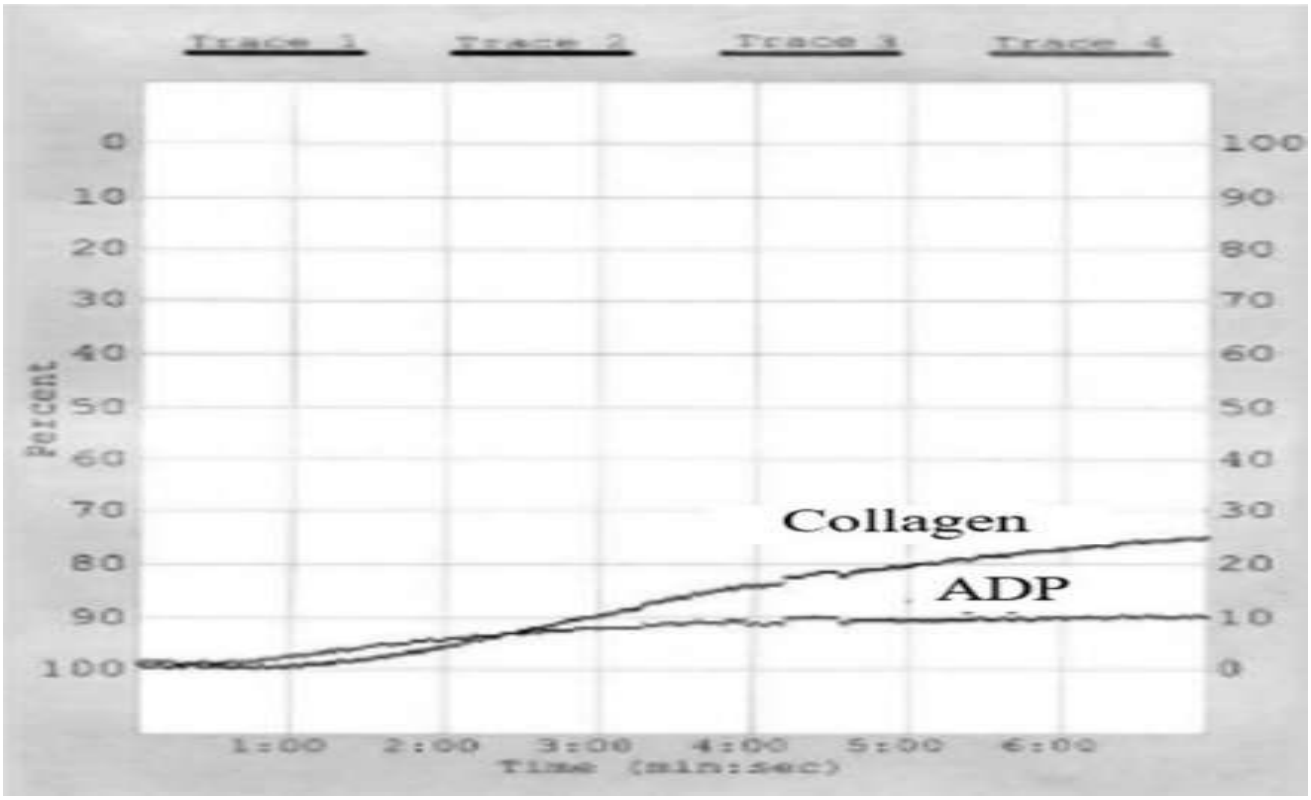


Figure 3: Response of the patient in Group III receiving combined ASA and clopidogrel therapy with reduction of platelet aggregation initiated by collagen and ADP

Comparing obtained results for patients from Groups I and III, significantly different conclusions were drawn. The differences in amplitude (12 and 5 ohm) and area under the aggregation curve (56.3 and 23.0 ohm*sec, respectively) for thrombocyte aggregation initiated by ADP were established by the median. For Group II (under clopidogrel intake) and Group (on combined therapy), no significant differences were found. The performed analysis between blood lipid spectrum, C-reactive protein, fibrinogen, and D- dimer on the one hand and three indicators of impedance aggregation on the other hand did not reveal any significant differences. Only slight correlation between the area under the aggregation curve in patients from Group II (clopidogrel) and C-reactive protein concentration (0.38, $p \leq 0.05$) as well as concentration of low-density lipoproteins (0.35, $p \leq 0.05$) was found. The results of electron microscopy showed the presence of leukocyte-platelet aggregates in the blood of patients at the inflammation process (Figure 4*b*). These units were found in every third IHD patient (32%).

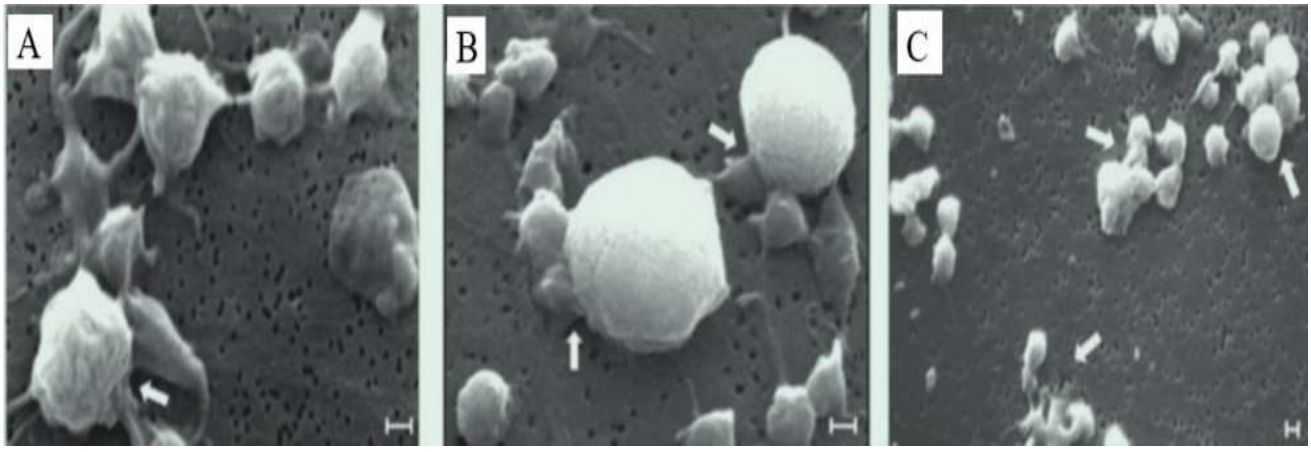


Figure 4: Thrombocytes of IHD patient at different magnifications: *a* – reticular platelet (bottom left), magnification 5000; *b* – aggregates of leukocytes and thrombocytes, magnification 5000; *c* – micro aggregates consisting of no more than 10-15 platelets, magnification is 2500

Such complexes include up to one-third of all leukocytes (34.1%). Besides, in patients with leukocytic thrombocyte aggregates in the blood, reticular platelets were found (Figure 4*a*), which are not common for healthy people and constitute up to 2% of the thrombocyte population in selected samples. These thrombocytes were found in 30% of patients. Due to the high hemostatic potential, reticulocyte platelets are more likely to form blood platelets (Figure 4*c*). Such aggregates are also absent in healthy people.

Discussion

The results of this research in the opposite correlation coefficients for Group I and Group II are expressed in the mechanisms of ASA and clopidogrel action. Since clopidogrel blocks P2Y12 receptors to ADP, there is no stimulus for the activation of platelet aggregation [9, 14]. ASA has no such effect on these receptors [15, 16], and, thus, no decrease in platelet aggregation associated with ADP has been reported.

Regardless of the cause for platelet action slowdown, they remain capable of receiving collagen signals through the GPIa receptor, but are unable to transmit this signal to the fibrinogen GPIIbIIIa receptors, which play a major role in cell aggregation between each other [17, 20]. The findings established allowed defining quantitative changes in the process of platelet aggregation by monotherapy with either ASA or clopidogrel, as well as a combined therapy. Moreover, the method of impedance aggregation enabled to determine what changes occur in relation to which inducers.

These data can be used as supporting information when comparing the effects of ASA and clopidogrel as antiaggregants. For example, in ASA monotherapy, the use of collagen as an inducer is more preferable due to its capability to prolong significantly the platelet aggregation time and reduce the area under the aggregation curve. Clopidogrel is considered to be more effective compared to ASA as for the changes it initiates in both the ADP and the collagen segment of thrombocyte aggregation, the use of two inducers in monotherapy with this drug is required.

During two major studies that included 5.955 and 13.847 patients with diagnosed acute coronary syndrome it has been established that timely clopidogrel therapy reduces the mortality rate in the hospital [21, 22]. Another study found that the abrupt shift from clopidogrel to ASA monotherapy for coronary syndrome and percutaneous coronary intervention contributed to a significant increase in myocardial infarction and lethal outcomes over the next three months [23]. At the same time, the high mortality rate remained for the next 6-9 months and more. This finding was confirmed by data from the UK, where continued clopidogrel monotherapy after discharge contributed to lower mortality among 7.543 patients [24].

A confirmed relationship between platelet activity initiation and adverse outcomes among patients during coronary stenting surgery has been stated [25, 28]. On the other hand, these data apply only to the

above case and are not applicable in the secondary prophylaxis or at stable patient status. Some researchers point out that clopidogrel therapy in coronary stenting is only required to suppress platelet initiation [29, 32]. At the same time, there is no proven evidence to support the increase in clopidogrel dosage for the above purposes. The GRAVITAS study [33] included 2.214 patients diagnosed with ischemic heart disease (60%) and acute coronary syndrome (40%).

Half a day or whole day after the implantation of the stent containing medication, the platelet activity level was detected. At that, patients received standard therapy with clopidogrel or ASA. In the case of high platelet activity, the loading dose of clopidogrel was increased to 600 mg, and the daily dose -to 150 mg. Other patients received normal doses of ASA and clopidogrel.

Six months later no cases of cardiovascular complications or mortality between the two study groups were reported. This could be due to the individual features of the patients. The data obtained through this study allows determining with high accuracy which dose of clopidogrel or ASA may initiate the platelet aggregation. Current research requires further investigation as it is necessary to confirm the data clinically on larger samples of patients, as well as to establish whether the studied platelet aggregation parameters are connected with undesirable cardiovascular events. Dosage correction of antiaggregant drugs may help to improve therapy efficacy and contribute to a more favorable prognosis.

Conclusions

The method of impedance aggregometry allows identifying and evaluating qualitative and quantitative changes that may occur in the aggregation of platelets with ADP and collagen as inducers. Thus, in ASA monotherapy, the use of collagen as an inducer results in prolongation of platelet aggregation period and reduction of area under the curve (correlation –0.80 ($p \leq 0.05$)). Clopidogrel monotherapy affects both, collagen and ADP, inducers.

In the case of ADP-initiated platelet aggregation, a positive correlation between amplitude and the area under the aggregation curve of 0.98 ($p \leq 0.01$) was recorded. A correlation of 0.77 ($p \leq 0.05$) was obtained between the platelet aggregation extension and the area under the curve at the collagen-induced process [34].

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